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Determination of Endogenous Norepinephrine Levels in Different Chambers of the Rat Heart by Capillary Electrophoresis Coupled with Amperometric Detection

Martin Novotny^a, Veronika Quaiserova-Mocko^a, Erica A. Wehrwein^b, David L. Kreulen^{b,*}, and Greg M. Swain^{a,*}

^a Department of Chemistry and the Neuroscience Program, Michigan State University, East Lansing, MI, 48824

^b Department of Physiology and the Neuroscience Program, Michigan State University, East Lansing, MI, 48824

Abstract

Capillary electrophoresis with end-column amperometric detection (CE-EC) was used to determine the regional distribution of norepinephrine (NE) in the hearts of sympathetically innervated (control) and chemically sympathectomized rats. Key features of the method are (i) the sample preparation and clean-up step that involved the application of off-line solid phase extraction (SPE) with a 95% NE recovery and (ii) the use of a diamond microelectrode for detection. NE was quantified in the left and right ventricle, the ventricular septum, and the left and right atrium. The NE concentration in the atria was 3–5 times higher than in the ventricles and ventricular septum of control rats. Basal NE levels in the left and right ventricle and the ventricular septum were reduced to below the detection limit (0.034 µg/g tissue) in tissues treated with the neurotoxin, 6-hydroxydopamine (6-OHDA), while only a moderate reduction was observed in the left and right atrium. Importantly, the diamond microelectrode provided low and stable background current and low peak-to-peak noise ≤ 0.65 pA at a detection potential of +0.86 V vs Ag/AgCl. A reproducible electrode response was observed for multiple injections of tissue homogenates with minimal response attenuation due to electrode fouling.

Keywords

sympathetic nervous system; heart; norepinephrine; capillary electrophoresis with electrochemical detection

INTRODUCTION

The sympathetic innervation of the heart arises primarily from neurons whose cell bodies are located in the bilateral stellate ganglia. The post ganglionic fibers reach the heart along the adventitial surface of the great vessels and are distributed via an epicardial plexus to the

Corresponding authors: Greg M. Swain, Department of Chemistry, Michigan State University, 320 Chemistry Building, East Lansing, MI 48824-1322. Tel. (517) 355-9715 x229, Fax. (517)353-1793, Email. E-mail: swain@chemistry.msu.edu. David L. Kreulen, Department of Physiology, Michigan State University, 2201 Biomedical Physical Sciences Building, East Lansing, MI 48824-1320. Tel. (517) 355-6475 x1312, Fax. (517) 355-5125, Email. E-mail: dkreulen@msu.edu.

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sinoatrial node, the conducting system, the coronary vessels and the myocytes of all four heart chambers (Wallis, Watson et al., 1996). Chronic activation of the sympathetic nervous system (SNS) is a characteristic of cardiovascular disease that leads to elevated blood pressure through a combination of increased cardiac output and vasoconstriction. One means of assessing SNS activity is to measure the concentration of endogenous norepinephrine (NE). Monitoring NE levels in urine, plasma, and importantly, tissue can reveal pathologic changes in SNS function associated with a disease state.

A goal of our group is to better understand alterations in SNS pathophysiology that occur in cardiovascular disease caused by salt-sensitive hypertension. It is well established that both salt-sensitive hypertension and congestive heart failure are characterized by heightened sympathetic activity (Kaye and Esler, 2005). Indeed, several changes in SNS function with the disease state are possible including differences in innervation, nerve firing rates, NE efflux per firing event, and reuptake. For example, dysfunction of the SNS, specifically NE reuptake, has been implicated in several cardiovascular disorders including hypertension, heart failure, orthostatic intolerance and postural tachycardia (de Champlain et al., 1966; Eisenhofer et al., 1996; Julius and Valentini, 1998; Esler and Kaye, 2000; Patel et al., 2000; Robertson et al., 2000; Rumantir et al., 2000; Shannon et al., 2000; Rupp, 2001; Carson et al., 2002; Goldstein et al., 2002; Qin et al., 2002; Schroeder et al., 2002; Münch et al., 2005). Additionally, there is evidence that the myocardial content of NE is reduced in subjects with congestive heart failure due to functional denervation (Kaye and Essler, 2005). Much of the work to date, though, directed toward understanding the dysfunction has involved global measures (e.g., plasma or whole organ levels of NE) of sympathetic tone. Full understanding of any cardiac dysfunction in the disease state will be realized, in part, only through chamber-specific studies of NE and the norepinephrine transporter protein (NET) content.

There is evidence of regional or chamber-specific differences in sympathetic innervation density, NE levels, and NET expression and function in the heart (Liang et al., 1989; Dawson and Meldrum, 1992; Böhm et al., 1998; Li et al., 2004). Regional sympathetic denervation is also known to occur following a heart attack or ischemia (Liang et al., 1989; Dae et al., 1995; Eisenhofer et al., 1996; Julius, 1998; Esler and Kaye, 2000; Li et al., 2004). A related finding is the depletion of NE that occurs in all heart chambers following cardiac transplantation (Mohanty et al., 1986). In order to better understand SNS dysfunction in cardiovascular disease, it is important to have a reliable method for monitoring NE content, and its metabolites, both during disease development and in the chronic disease state (Gilinsky et al., 2001; Nikolajsen and Hansen, 2001; Berquist et al., 2002; Peaston and Weinkove, 2004; Makoto, 2006).

There is great interest in developing highly sensitive analytical techniques that can be used to distinguish biogenic amines and their metabolites with a high degree of resolution (Paxon et al., 2005). HPLC with electrochemical detection is the most commonly used method for quantitative determination of biogenic amines and metabolites in tissue, plasma and urine (Davis et al., 1981; Cheng and Kuo, 1995; Chi et al., 1999; Gilinsky et al., 2001; Unceta et al., 2001; Sastre et al., 2004; Sabbioni et al., 2004; Patel et al., 2005; Machida et al., 2006). Another informative, but less used technique, for bioanalyte monitoring in tissue is capillary electrophoresis (CE) (Dillon and Sears, 1998; Qurishi et al., 2002; Markuszewski et al., 2003; Shou et al., 2004; Paxon et al., 2005). This is primarily because of (i) the complexity of tissue samples, which can require extensive sample preparation and clean-up steps in order to facilitate isolation and identification of the target analyte(s) and (ii) the high concentration of perchloric acid that is often used for tissue homogenization. CE, employing narrow inner diameter capillaries, enables sampling from small volumes, is relatively simple and allows rapid analysis times with high resolving power (Paxon et al., 2005). The technique is increasingly being adopted in clinical analysis because of its ability to separate a wide variety of molecules with high efficiency, ranging from small inorganic ions and organic molecules

to large biomolecules (Kuhr and Monning, 1992; Lehman et al., 1997; Thormann et al, 1999; Cvačka et al., 2003; Makoto, 2006). CE possesses the highest resolving power of any liquid separation technique and offers distinctly different selectivity, as compared to HPLC (Isaaq, 2000). Additional benefits include versatility, low operating cost and reagent consumption, and the ability for on-column analyte preconcentration using sample stacking (Kuhr and Monning, 1992; Urbánek et al., 2003). A recent review highlights advances that have been made in the application of CE to neuroscience, in particular, the monitoring catecholamines, amino acids, proteins, peptides and nucleic acids (Powell and Ewing, 2005).

Herein, we report on the application of a CE-EC method for determining the regional distribution of NE in the hearts of sympathetically innervated (control) and chemically sympathectomized rats. NE levels in the left and right atria, left and right ventricle and the ventricular septum are reported. Key features of the method are (i) an optimized solid phase extraction (SPE) procedure for the sample clean-up and (ii) the use of a diamond microelectrode for sensitive, reproducible and stable end-column electrochemical detection.

EXPERIMENTAL

CE-EC

The CE system with end-column amperometric detection was constructed in-house and has been described previously (Cvačka et al., 2003; Muna et al., 2005). Briefly, the instrument consisted of a 30-kV variable power supply (Spellman, Hauppauge, NY), an electronic timer relay for the electrokinetic injection and a plexiglas box with a safety interlock housing 76 cm long $\times$ 29 μ m i.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ). End-column amperometric detection was performed in a glass detection cell using a three electrode arrangement consisting of a boron-doped diamond microelectrode, an Ag/AgCl reference electrode (a 4 M KCl saturated with AgCl filling solution, Fisher Scientific, Hampton, NH) and a platinum counter electrode in a glass detection cell (Cvačka et al., 2003). The working electrode was positioned at the end of the separation capillary at a distance estimated to be about 10–20 μ m. The electrodes were connected to a potentiostat (Model 832A, CH Instruments, Inc., Austin, TX) and electropherograms were acquired using the instrument software for *i-t* measurements. The separation capillary was conditioned prior to use by sequentially passing 0.1 M sodium hydroxide, purified water and run buffer for 15 min each. Separation was performed using a 250 mM boric acid/KOH run buffer at pH 8.8, and a separation voltage of +24 kV (316 V/cm). The voltage was applied between the run buffer reservoir at the capillary inlet (anode) and an electrically-grounded run buffer reservoir at the column outlet (cathode). The analyte sample was electrokinetically injected at +18 kV for 8 s. The injection volume, using methanol as a marker, was determined to be 6 nL (Huang et al., 1988). A multistep SPE procedure, described below, was used to prepare the tissue homogenate for injection.

Boron-Doped Diamond Microelectrode Fabrication and Characterization

Boron-doped diamond thin film was deposited on a sharpened, 76 μ m diameter Pt wire by microwave-assisted chemical vapor deposition (1.5 kW, 2.54 GHz, ASTeX, Woburn, MA), as described elsewhere (Cvačka et al., 2003; Park et al., 2005, 2006, 2006a). The deposition conditions were as follows: a 0.5 % CH₄ in 99.5 % H₂ source gas mixture containing 10 ppm diborane (B₂H₆ diluted in H₂) for doping, a system pressure of 45 Torr, a substrate temperature of ~700 °C as estimated with an optical pyrometer, a microwave power of 400 W, and a total gas flow of 200 sccm. All source gases were ultra high-purity grade, 99.999%. The deposition time was 10 h resulting in a film thickness of ca. 5 μ m (Cvačka et al., 2003; Park et al., 2005, 2006, 2006a). Fabrication of the microelectrode was completed by attaching the diamond-coated Pt wire to the end of a 6-cm long copper wire that was 0.2 mm in diameter (Goodfellow

Cambridge Ltd., Huntingdon, England), using conductive silver epoxy (CW2400 Chemtronics, Kennesaw, GA). The epoxy was cured overnight in air at room temperature. The microelectrode was then insulated by insertion into a small polypropylene pipette tip (0.5 – 10 μ L tip, Daigger, Vernon Hills, IL) followed by heating the end to melt the polymer (Park et al., 2006, 2006a). This caused the polymer to conformally coat the diamond electrode with a tightly bound insulation layer. This procedure produced a conically shaped electrode with an exposed length of ~200 micrometers. Each fabricated diamond microelectrode was characterized by cyclic voltammetry, prior to use, using several common redox systems including 1 mM $\text{Fe}(\text{CN})_6^{-3/-4}$ in 1M KCl, (Cvačka et al., 2003; Park et al., 2005).

Animals

Five adult male, Sprague-Dawley rats, 8 weeks old (Charles River, Portage, MI) weighing 250–275 g were used in this study. All animal experiments were performed in accordance with the “Guide for the Care and Usage of Laboratory Animals” (National Research Council) and were approved by the Animal Use and Care Committee of Michigan State University. The animals were randomly divided into control (untreated) and denervated groups (see details in the following section). Animals of the same treatment group were housed two per cage in a temperature and humidity-controlled room using a 12 h light/dark cycle. Standard pellet rat chow and water were given *ad libitum*.

Sample Preparation

1. Tissue collection—Rats were anesthetized with a lethal dose of sodium pentobarbital (65 mg/kg, intra-peritoneal) followed by thoracotomy. Hearts were removed and immediately placed into chilled (4 °C) phosphate buffered saline solution containing 137 mM sodium chloride, 2.7 mM potassium chloride, 4.3 mM sodium phosphate dibasic and 1.4 mM potassium phosphate monobasic. While in the buffer, the chambers were quickly separated in the following order: right atrium, left atrium, right ventricle outer wall, ventricular septal wall, and left ventricle outer wall. Dissected chambers were immediately frozen by contact with dry ice and stored at –80 °C until further processing. Proper storage of tissue samples after isolation (i.e., avoidance of oxidative degradation) is critical for accurate measurement of catecholamines (Zhang et al., 2001).

2. Tissue sample homogenization—A frozen piece of tissue was weighed and homogenized using a variable speed Omni TH-115 homogenizer with a 5 mm diameter EZ-Gen generator probe (Omni International Inc., Warrenton, VA) in ice-cold 0.1 M perchloric acid. For the left and right ventricles and the ventricular septum, 2 mL/0.5 g tissue was used, and for the right and left atria, 13 mL/0.5 g tissue was used. The tissue was homogenized for ~ 3 min at 35,000 rpm. The homogenate was then centrifuged at 10,000 rpm for 15 min at 4° C. Using a pipette, the supernatant was carefully drawn off without disturbing the precipitate.

3. Solid phase extraction (SPE)—A modified generic method for extraction of basic compounds was used for NE isolation from the supernatant. SPE was performed using an Oasis MCX cartridge (Waters, Milford, MA, USA) with 30 mg of sorbent in 1 mL syringe barrel. The solid phase had a 30 μ m particle size and an 80 Å pore size. NE was retained on the solid phase by two mechanisms: π - π interactions and cation exchange. The extraction was performed using a 12-port vacuum manifold (Alltech Associates, Inc., Deerfield, IL). The sorbent was conditioned prior to use by passing 1 mL of methanol followed by 2 mL of ultrapure water. Then, 0.7 – 1 mL of the sample supernatant in perchloric acid was loaded onto the solid phase and passed through at a flow rate < 0.5 mL/min (contact time of 4–5 min). The sorbent bed was then washed with 2 mL of 0.1 M hydrochloric acid followed by 1 mL of methanol. The sorbent was then vacuum-dried for 6 min prior to analyte elution. Elution was accomplished by passing the CE run buffer, using the same volume as the sample loading (4–5 min at < 0.5

mL/min). Therefore, the preconcentration factor was 1. The collected eluate was directly injected onto the CE column for analysis.

Denervation with 6-OHDA

Chemical sympathetic denervation was accomplished with the neurotoxin, 6-hydroxydopamine (6-OHDA). Systemic administration of 6-OHDA produces long-lasting depletion of NE from sympathetically innervated organs, including the heart (Thoenen and Tranzer, 1968, 1973). 6-OHDA was prepared fresh as a mixture of 0.9 % (154 mM) sodium chloride and 0.5 % (28.4 mM) ascorbic acid before application and kept in the dark. Animals were dosed according to body weight (250 mg/kg) with a subcutaneous injection of the neurotoxin. The drug was administered with a 25 ga. needle to the loose skin between the shoulder blades using 3 consecutive injections during a one-week period on days 1, 3 and 5. On day 7, two days after the final dose, the animals were sacrificed as described above. Control animals were age and sex matched to the treated group, but did not receive a 6-OHDA injection.

Materials and Chemicals

Norepinephrine (NE), 6-hydroxydopamine hydrobromide (6-OHDA) and 5-ethyl-5-(1-methylbutyl)-2,4,6-pyrimidinetrione sodium salt (sodium pentobarbital) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Chemicals used in the run buffer were boric acid (Sigma-Aldrich Co.) and potassium hydroxide (Columbus Chemical Industries, Columbus, WI). The run buffer was filtered through a 0.4 μ m nylon membrane filter (Sigma-Aldrich Co.) before use. Sodium hydroxide and sodium metabisulfite were obtained from Spectrum Quality Products, (Gardena, CA). Tissue homogenization was performed in 0.1 M perchloric acid (ultrahigh purity, 99.999%, Sigma-Aldrich Co.). A stock solution of NE (10 mM) was prepared with 100 mg of sodium metabisulfite, 800 mg of sodium chloride (Jade Scientific, Canton, MI) and 500 μ L of 37% hydrochloric acid (ultrahigh purity, 99.999%, Sigma-Aldrich Co.), all dissolved in 100 mL of ultrapure water (Raggi et al., 1999). Lower analyte concentrations were prepared in the run buffer. HPLC grade methanol (Sigma-Aldrich Co., St. Louis, MO) was used for SPE extraction. Ultrapure 18 M Ω water (Barnstead E-Pure System, Barnstead International, Dubuque, IA) was used for all solution preparation. All chemicals were reagent-grade quality, or better, and were used as received.

RESULTS AND DISCUSSION

The first task in the method development was the determination of the figures of merit for NE from standard solutions using the CE-EC method with offline SPE. Reproducible separations, in terms of the migration times and peak shapes, were achieved in a 250 mM boric acid/KOH, pH 8.8, run buffer. The separation of NE from other catecholamines and metabolites present in tissue in this run buffer was reported previously (Park et al., 2006a). NE migrates as a negatively charged compound in this buffer by virtue of its complexation with borate (Wallingford and Ewing, 1988; Landers et al., 1992). Hydrodynamic voltammograms for NE were initially recorded in order to determine the optimum detection potential (data not shown). Sigmoidally-shaped *i*-*E* curves were obtained with mass-transfer limited response seen at potentials around 0.95 V. An example electropherogram for a left atrium homogenate is shown in Figure 1. The trace, recorded at 0.95 V, has an intense peak for NE with a migration time of 519 s. At this potential, though, the peak for NE is convoluted with at least two additional co-migrating solutes. There are also a number of other intense peaks for as yet unidentified compounds in the 300 to 600 s time window. Improved selectivity for NE and reduced interference by the unidentified peaks was achieved by lowering the detection potential to 0.86 V, as shown in the bottom trace in Figure 1. The interfering peak intensities were reduced in magnitude, thus enabling more accurate NE identification and quantification, albeit with some loss in response magnitude. The slight decrease in response sensitivity was more than made

up for by the improved selectivity. The peak for NE, which was identified by migration time matching by spiking with an NE standard, is present at both detection potentials.

Using the lower detection potential and the borate run buffer, the detection figures of merit for NE were determined using a series of standards and are summarized in Table 1. A linear dependence of the peak area and height with the NE concentration was found in the range of 0.062 – 1 μ M with a correlation coefficient of 0.999. The LOD was obtained from the calibration plot using the relationship, $LOD = 3 \times \text{noise/slope}$. The concentration LOD was determined to be 51 nM ($S/N=3$), which corresponds to a mass LOD of 0.05 pg (The United States Pharmacopeia, 1995). The baseline noise (i.e., peak-to-peak current) at the detection potential was 0.65 pA, or less. The response reproducibility for a series of 5 NE injections (0.5 μ M) was 2.58 % (RSD) for peak height and 3.35 % for peak area. The height equivalent to a theoretical plate (HETP) for NE was calculated to be $17.6 \pm 4.4 \mu\text{m}$. These detection figures of merit compare favorably with those previously reported for standard solutions using the CE-EC method without SPE (Park et al., 2006a).

Next, the method was applied to the measurement of NE in heart tissue. The particular chamber was homogenized, as described above, and the resulting supernatant was subjected to SPE for sample clean-up prior to separation and detection by CE-EC. Preconcentration is also possible with SPE but, in this work, none was used. As indicated above, the preconcentration factor was 1. High NE recovery of $95.1 \pm 5.6 \%$ ($n=6$) was achieved with the solid phase (Quaiserova-Mocko et al., 2006). NE was loaded as a cation and thus retained on the solid phase by a combination of cation-exchange and π - π interactions. As mentioned above, elution with the boric acid buffer provided good selectivity for NE because of the formation of a negatively charged complex with borate at pH 8.8. A typical electropherogram of left atrium homogenate was presented in Figure 1. The electropherograms for the left and right atrium homogenates were similar in terms of the peaks present, their migration times, and their peak shapes so only the curve for the left atrium is shown. Figure 2 compares electropherograms for left ventricle and ventricular septum homogenates. As was the case for the atria, the electropherograms for the ventricles were similar in terms of the peaks present and their migration times. The NE peak at 519 s is evident in both electropherograms along with intense and unidentified peaks between 350 and 400 s, and between 550 and 650 s. It is interesting that these peaks are absent in the electropherogram for the left atrium (Fig. 1) at 0.86 V. Attempts to identify the unknown peaks through migration time matching with various standard catecholamine and metabolite solutions proved unsuccessful. For example, we verified that none of these peaks are attributable to 5-hydroxytryptamine (serotonin, 5-HT), α -(aminomethyl)-4-hydroxy-3-methoxy benzenemethanol (normetanephine, NMN) or dopamine as these compounds were not eluted from the solid phase with the boric acid buffer. Furthermore, none of the peaks are associated with the catecholamine precursor, L-DOPA, or the NE metabolites, 3,4-dihydroxyphenylethyleneglycol (DOPEG) and α -4-dihydroxy-3-methoxy-benzeneacetic acid (vanillylmandelic acid, VMA) or dopamine metabolite, 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), because they all exhibit migration times between 800–900 s, as shown in (Park et al., 2006a).

A four-point standard addition protocol was used to quantify NE in the different heart chambers of the control rats and a summary of the results is presented in Figure 3. Data are reported for three control rats and values are expressed as μg of NE per g of frozen tissue. Each value was calculated as an average from 4 measurements at the 95% confidence interval. The vertical bars represent the relative standard deviation. The LOD was determined to be 0.034 $\mu\text{g/g}$ tissue for the ventricles and ventricular septum, and 0.22 $\mu\text{g/g}$ tissue for the atria. This difference is due to the factor of ~ 6 difference in volume of perchloric acid used for the tissue homogenization. It can be seen that the highest nominal NE concentrations were found in the atria, specifically the right atrium. The nominal values range from 1.40 to 1.90 $\mu\text{g/g}$ tissue for

the left atrium and from 1.80 to 2.90 $\mu\text{g/g}$ tissue for the right atrium. Lesser concentrations from 0.20 to 0.80 $\mu\text{g/g}$ tissue were found in the left and right ventricles and the ventricular septum. Clearly, the NE concentration decreases in order of right atrium > left atrium >> left ventricle, right ventricle and ventricular septum.

Systemic administration of 6-OHDA produces long-lasting depletion of endogenous NE from peripheral sympathetically innervated organs (Thoenen and Tranzer, 1968, 1973). In order to better understand the role of SNS dysfunction in cardiovascular disease, the effect of a 6-OHDA induced sympathectomy on NE levels in the heart was studied. This study served two purposes. First, it provided evidence that NE was in fact being measured in the innervated tissues. Second, it served to simulate the disease states for which lower NE concentrations exist (Petch and Nayler, 1979; Mohanty et al., 1986; Dae et al., 1995; Eisenhofer et al., 1996; Julius, 1998; Esler et al., 2001; Li et al., 2004). Example electropherograms of right ventricle homogenate from control (top line) and 6-OHDA sympathectomized rats are shown in Figure 4. No peak is observed at the NE migration time of 519 s in the electropherogram for the chemically sympathectomized tissue. The results for the neurotoxin-treated rats are also summarized in the table presented in Figure 3. 6-OHDA treatment reduced the NE concentration in the left ventricle, right ventricle and ventricular septum to below the LOD, but only reduced NE in the right atrium by 25% to ca. 1.60 $\mu\text{g/g}$ tissue and in the left atrium by 33% to ca. 1.20 $\mu\text{g/g}$ tissue; well above the LOD. This pattern is in agreement with previous findings in guinea pig heart where 6-OHDA reduced the NE concentration in the atria less than in the ventricles (Bentley et al., 1976). Since the effectiveness of the neurotoxin depends on reuptake into the nerve terminal, it could be that expression of NET differs in atrial versus ventricular neurons. This remains for further study. In support of this supposition, however, is the fact that recent immunohistochemical and western blot data have confirmed the presence of NET protein in sympathetic fibers in the heart, and have suggested that NET protein levels are inversely related to NE content of the chambers, with the atria containing less NET than ventricles (Wright et al., 2006).

Several important points about the method can be made. First, the CE-EC method coupled with the SPE procedure is a straightforward and reliable approach for NE analysis in cardiac tissue. The method is particularly well suited for cases in which the sample volume is limited. Higher mass limits of detection should be possible as compared to conventional HPLC. It should be possible to expand the scope of the method to include other important bioanalytes and metabolites. Furthermore, performing the separation in the micellar electrokinetic chromatography (MEKC) mode would provide an additional measure of speciation for uncharged solutes and solutes with similar charge-to-size ratio. Second, the method was validated against a standard reversed-phase HPLC separation with both amperometric and coulometric detection (Quaiserova-Mocko et al., 2006). With regard to chamber-specific concentrations on NE, excellent agreement was seen in the results obtained by CE-EC and LC-EC (both amperometric and coulometric detection modes). Third, the method benefits from the excellent properties of the diamond electrode. Diamond provides as good or superior detection figures of merit for NE, as compared to carbon fiber, and possesses the additional benefits of (i) negligible electrode fouling in the presence of the tissue homogenates and (ii) requires no time-consuming pretreatment for activation. Fourth, the chamber-specific concentrations of NE reported herein are in good agreement with those previously reported in the literature for either whole rat hearts or for specific chambers. For example, Yamada and coworkers reported nominal values of approximately 1.6 and 1.1 $\mu\text{g/g}$ tissue for the right and left atrium, 0.6 and 0.4 $\mu\text{g/g}$ tissue for the right and left ventricle, and 0.3 for the ventricular septum. After treatment with 6-OHDA, there was a 60–80% reduction in NE levels in all chambers. The approximate concentrations in the right and left atrium decreased to 0.35 $\mu\text{g/g}$ tissue, in the right and left ventricle decreased to 0.1 to 0.25 $\mu\text{g/g}$ tissue and in the ventricular septum decreased to 0.15 $\mu\text{g/g}$ tissue (Yamada et al., 1980). Eldrup and coworkers reported a

whole heart NE concentration between 0.65 to 0.85 µg/g tissue in control rats (Eldrup et al., 1989). Lear and Prohaska noted a mean atrial NE concentration of 2.3 µg/g tissue and a mean ventricular concentration of 1.5 µg/g tissue for control rats (Lear and Prohaska, 1997). Finally, Kawamura et al. reported a mean whole heart NE concentration of 1.0 µg/g tissue for the control and a decrease of 93% to 0.07 µg/g tissue after 6-OHDA treatment (Kawamura et al., 1999).

CONCLUSIONS

It has been shown that capillary electrophoresis with electrochemical detection can be used to reliably measure normal and pathologically low levels of NE in different chambers of the rat heart. Keys to the method are the use of a diamond microelectrode, which provided superb response sensitivity, reproducibility and stability, and a sample preparation method that utilized off-line solid phase extraction. The NE concentration in the control animals decreased in the following order: right atrium > left atrium >> left and right ventricle and ventricular septum. The LOD was determined to be 0.034 µg/g tissue for the ventricles and ventricular septum, and 0.22 µg/g tissue for the atria (S/N=3). Treatment of the rats with 6-OHDA produced a marked reduction in NE in the left and right ventricle and the ventricular septum. Only slight decreases in NE content were found in the left and right atrium. The results demonstrate that the method is useful for assessing regional NE concentration differences in the heart and is presently being used in our laboratory to elucidate changes in sympathetic neural control associated with salt-sensitive hypertension.

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Figure 1.

Electropherogram for a left atrium homogenate from a control rat recorded at a detection potential of 0.95 V (top curve) and 0.86 V vs Ag/AgCl (bottom curve). CE conditions: 29 μm i.d. $\times$ 76 cm fused silica capillary, 250 mM boric acid/KOH buffer, pH 8.8, applied voltage 24 kV, electrokinetic injection 18 kV/8s.

Figure 2.
Comparison of electropherograms obtained for left ventricle (top) and ventricular septum (bottom) homogenates from a control rat. CE-EC conditions: 29 μm i.d. $\times$ 76 cm fused silica capillary, 250 mM boric acid/KOH buffer, pH 8.8, applied voltage 24kV, electrokinetic injection 18 kV/8s, detection potential +0.86 V vs Ag/AgCl.

Figure 3.

Quantitation of norepinephrine by CE-EC in heart tissue compartments, left ventricle (LV), ventricular septum (VS), right ventricle (RV), left atrium (LA) and right atrium (RA). Columns represent values for rats 1, 2 and 3 of the control group (patterned columns) and rats 4 and 5 of the 6-OHDA treated group (solid columns). Values are expressed as µg of NE per g of frozen tissue (±RSD, vertical bar).

Figure 4.

Comparison of electropherograms for right ventricle homogenates from a control (top curve) and a chemically sympathectomized (bottom curve) rat. The inset shows an enlarged view of 450–600 s time window. CE-EC conditions: 29 μm i.d. $\times$ 76 cm fused silica capillary, 250 mM boric acid/KOH buffer, pH 8.8, applied voltage 24kV, electrokinetic injection 18 kV/8s, detection potential +0.86 V vs Ag/AgCl.

Table 1

CE-EC Separation and Amperometric Detection Figures of Merit for Norepinephrine.

Concentration range, μM	0.062 – 1.0
Linear dynamic range, μM	0.062 – 1.0
Slope (sensitivity) ^b , $\mu\text{A.s.L.mol}^{-1}$	322 ± 5
Absolute sensitivity, $\mu\text{A.s.ng}^{-1}$	54.4
Intercept ^b , pA.s	-1.89 ± 2.56
Standard error, pA.s	3.79
Correlation coefficient	0.999
Concentration limit of detection, μM	0.051
Mass limit of detection, pg	0.050
HETP, μm	17.6 ± 4.4

^a) CE-EC parameters: separation buffer = 250 mM boric acid/KOH, pH 8.80; capillary = 29 μm i.d $\times$ 76 cm length; $E_{\text{sep}} = 24\text{kV}$; $E_{\text{inj}} = 18\text{kV}$; $t_{\text{inj}} = 8\text{s}$; $E_{\text{det}} = +0.86\text{ V}$ vs. Ag/AgCl. The limit of detection expressed as an absolute mass (injected concentration $\times$ apparent injection volume). $\text{LOD} = (3 \times \text{noise})/\text{slope}$. The HETP value was determined from 4 measurements using a solution of 0.5 μM norepinephrine (95% confidence interval). Mean and standard deviation values are given for some parameters.